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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF EXTRACTS FROM SEEDS OF *MUSA BALBISIANA*

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ABSTRACT

In this paper, some extracts from seeds of *Musa balbisiana* were prepared with some solvents such as petroleum ether, ethyl acetate and buthanol. The results showed that the microwave-assisted extraction had high yield. The buthanol and ethanol extracts showed good antioxidant activity (using reaction with free radical DPPH, 1,1-diphenyl-2-picrylhydrazyl) with IC_{50} value of 182.6 and 301.8 µg/mL, respectively. The minimum inhibitory concentration *MIC* of buthanol extract was 22.5 mg/mL with both *Enterococcus faecalis* and *Staphylococcus aureus*. Besides, the ethyl acetate extract had *MIC* value of 7.5 and 3.5 mg/mL for *Enterococcus faecalis*and *Staphylococcus aureus,* respectively. From results of this study, these extracts are promising for researches as well as applications in the future.

Keywords: seeds of Hot banana, *Musa balbisiana*, antioxidant, IC_{50}, antibacteria, MIC.

1. INTRODUCTION

Banana is one of the famous fruitsthatis used worldwide for nutritional and medicinal purposes. It is considered a wonderful source of potassium and essential vitamin. Traditional remedy also used banana for treatment of diarrhea, gastric ulcers and some other health troubles. Nowadays, many researchers have discovered and proved excellent biological activities of bananas [1].

Many species of banana exist in the world and they have quite different biological properties. However, there is only little information about the activities of the different parts of such fruits. Most publications just focused on pulps or whole fruits. Interestingly, some authors found that the peel and seed fractions showed higher antimicrobial and antioxidantactivity than the pulps [2, 3].

Musa balbisiana - Pip banana (chuối hột) is a wild, seedy banana native to South and South East Asia including Vietnam. In Vietnamese traditional medicine, *Musa balbisiana* is used to

reduce of diabetes, kidney stones and cancers. Several studies have been taken place in Vietnam, in order to investigate chemical constituents as well as biological properties of Pip banana. For example, Tran Van Sung isolated cyclomusalenon [(24S)-24 methyl-29-norcycloart -25-en-3-on] and stigmasterol from Pip banana [4]. Do Quoc Viet found that total extract from Pip banana fruits could reduce blood glucose index on mice [5]. Bui My Linh & co-workers investigated some pharmacological activities and toxicity of Pip banana. In 2002, Bui My Linh had one paper in which she isolated some low-polarity substances from seeds [6, 7]. This is one of a little publications focused on seeds of Pip banana. Hence, main objective of this paper is to evaluate phytochemical screening and antioxidant and antibacterial activities of some extracts from the Pip banana seeds. The success of this study could open new research direction for preparing pharmacological extracts in application on manufacturing functional foods as well as pharmaceuticals.

2. EXPERIMENTS

Seeds of Pip banana (*Musa balbisiana)* was a commercial product and purchased from BinhThoi market (Hochiminh city, Vietnam) on March, 2014. It was ground to fine powder and stored in a closed container. Buthanol (BuOH), ethyl acetate (EtOAc) and petroleum ether (PE) were supplied by Xilong (China). The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and vitamin C was given from Sigma Aldrich. All chemicals for phytochemical screening tests were used without further purification.

Firstly, a 50 g powder of banana seeds was extracted with solvents (300 mLx 2 times) for 120 minutes at reflux condition. After filtered, all filtrates were mixed together and the solvent was removed by using rotating evaporator to collect total extract. The extraction was repeated with different solvents as petroleum ether, ethyl acetate and buthanol, respectively. In investigation of microwave effects, the extraction was carried out in a modified microwave oven for 30 minutes (Sony…).

The water and ash contents were determined by methods in Vietnamese pharmacopoeia IV [8]. Phytochemical screening examinations were conducted to identify the phytochemical constituents according to standard methods. Yield of extract was calculated by weight ratio of total extract and using material. Antioxidant activity was conducted by a reaction with the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [9, 10] and using vitamin C as a control. The antibacterial assays were carried out by microdilution method with bacterial inoculum about 10^4 cfu/well. The lowest concentrations of the extracts in the microplates with no growth were used to determine the minimum inhibitory concentrations (MICs). The tests were carried out on *Staphylococcus aureus* ATCC 29212 and *Enterococcus faecalis*at University of Medicine and Pharmacy - Ho Chi Minh city.

3. RESULTS

In Vietnam, the seeds of Pip bananas were very popular and available in the market. They were prepared by using naturally ripened banana fruits, removing peels and pulps and drying, that the seeds have dark color, quite round shape and diameter about 4-5 mm. After ground, the powder had light grey and the water and ash contents were 12.4 % and 3.9 %, respectively (Figure 1).

Extracting condition and solvents affected to resulted yield. Ethyl acetate and buthanol had higher yields of 1.32 % and 2.68 % in supporting of microwave (comparing that of 0.99 % and

1.35 % by using the water bath). Because of high polarity, ethyl acetate and buthanol are affected by microwave easily and they take out more substances. Contrarily, petroleum ether with very low polarization had low yield of 0.59 % in microwave condition (0.73 % in traditional condition). As the results, total extracting yield was 3.07 % and 4.59 % when using water bath and microwave oven, respectively (Figure 2).The total extracting yields with above solvents were very low because the seeds consisted of high ratio of pulp and carbohydrates and organic solvents could not pull them out. In all next steps, the extracts with supporting of microwave were used to investigate.

(a) (b)

Figure 1. Seeds of Pip banana (a) and ground powder (b).

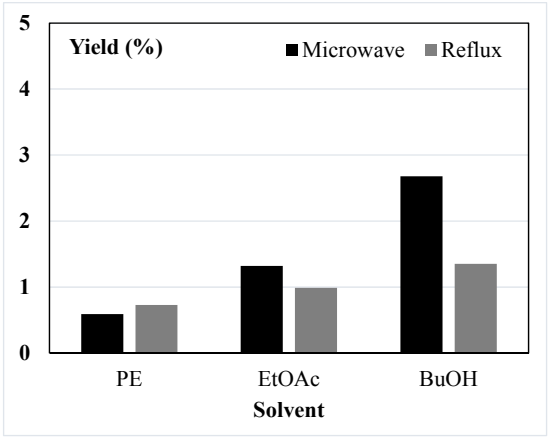

Figure 2. Effect of extracting condition.

The phytochemical screening tests are shown in Table 1. In the results, the EtOAc contained flavonoids, phenols and glycosides. Besides, the BuOH extract also had similar constituents, and free acids and glycosides presented in the petroleum ether extract.

Table 1. Phytochemical screening of extracts from *Musa balbisiana.*

Compounds	**Reagent/test**	**Extracts**		
		Petroleum ether	**Ethyl acetate**	**Buthanol**
Flavonoids	Alhaline reagent	–	+ +	+
Phytosterols	LibermannBurchard' s test	–	–	–
Carotenoids	H_2SO_4 test	–	–	–
Alkaloids	Mayer's reagent	–	–	–
Acids	Na_2CO_3 test	+	+	–
Reducing sugars	Fehling's test	–	–	–
Saponins	Foam test	–	–	–
Tanins& phenols	$FeCl_3$ test	–	+ +	+
Glycosides	Keller-killiani test	+	+	+ +
Fats & fatty acids	Spot test	–	–	–
(+: presence; -: absence)				

The results showed that the ethyl acetate and buthanol extracts have large classes of phyto-constituents, which may be responsible for many pharmacological activities. The flavonoids and phenols are constituents that have high antioxidant capacity. Moreover, the flavonoids act as an

anti-inflamatory agent. Solvents used for extraction significantly affected the amount of flavonoids and phenolics obtained in the extracts. It seemed that ethyl acetate could be a good solvent (Table 1) with visible and strong detections.

In this study, the antioxidant property was determined by using DPPH because of effective and popular method. Similar to above investigations, the polarities of solvents also affected to the antioxidant activity. The highest scavenging activity was observed with the BuOH extract with IC_{50}value of 182.6 mg/mL. That was followed by the EtOAc extract with IC_{50} value of 301.8 mg/mL and the PE extract exhibited the worst activity with IC_{50} of 1551 mg/mL (Figure 3). Researches from Preeti Jain and co-workers found that the EtOAc and hexane seed extracts of *Musasapientum* banana in Bangladesh had antioxidant activity with ascorbic acid equivalentantioxidant capacity value of 1238.33 and 741.4 mg AA/ 100 g extract, respectively [2]. High phenolic and flavonoid contents could lead to high antioxidant activities. The results indicated that ethyl acetate and buthanol could be good solvents for extracting antioxidant substances from banana seeds. Their studies also exhibited that solvent with low polarity (hexane or petroleum ether) was not suitable for this purpose.

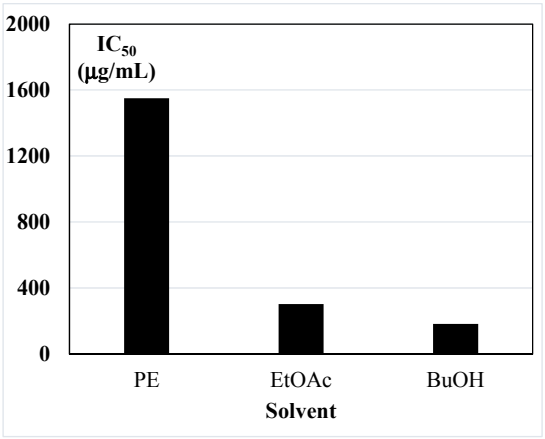

Figure 3. Theantioxidant activity with IC_{50} value of extracts from *Musa balbisiana.*

Antibacterial activity of all extracts of seeded banana was evaluated against two Gram positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus* by disc diffusion method. They are common cause of human infections. Moreover, *Enterococcus faecalis*is resistant to many commonlyusedantimicrobial agents,so a new antibacterial product is nescessary. The results obtained are presented in Table 2. Generally, the EtOAc had the best activity with MIC value of 7.5 and 3.75 mg/mL on both bacteria. Unfortunately, the petroleum extract exhibited no effects on concentration range of 0.02 - 10 mg/mL and the minimum concentration to inhibit bacteria may higher than 10 mg/mL. The minimum inhibitory concentration MIC of buthanol extract for both bacteria was 22.5 mg/mL. On another seedy banana (*Musa sapientum* L), the EtOAc seed extracts also had the best antibacterial activity against *Staphylococcus aureus* comparing to hexane and ethanol extracts. Incase of EtOAc extract obtained from seeds, the MIC value was found to be 6.5 mg/mL against *S. aureus* [2]. Obviously, the EtOAc extract from Pip banana seeds had stronger antibacterial activity.

Based on above results, it can be said that seeds of Pip banana possess significant antibacterial and antioxidant activities. Specially, the ethyl acetate extract showed as the best product. The above phytochemical test found this extract having flavonoids and polyphenols with high quantity. It could be basic evident for further researches to isolate and identify actives presenting in the extracts and possibly exploited for pharmaceutical uses. Moreover, the seeds can be further screened against various bacteria and diseases in order to find out its activity. From results of this study, the EtOAc extracts are promising for researches as well as applications in the future.

Table 2. Antibacterial activity of extracts from *Musa balbisiana.*

	Control DMSO 10%	Concentration (mg/mL)									
		Petroleum ether extract									
		10	5	2.5	1.25	0.63	0.31	0.16	0.08	0.04	0.02
E. faecalis	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+
		Ethyl acetate extract									
		15	7.5	3.75	1.88	0.94	0.47	0.23	0.12	0.06	0.03
E. faecalis	+	-	-	+	+	+	+	+	+	+	+
S. aureus	+	-	-	-	+	+	+	+	+	+	+
		Buthanol extract									
		45	22.5	11.25	2.81	1.41	0.7	0.35	0.18	0.09	0.04
E. faecalis	+	-	-	+	+	+	+	+	+	+	+
S. aureus	+	-	-	+	+	+	+	+	+	+	+
(+: visible growth; -: no visible growth)											

4. CONCLUSIONS

There has been an increasing demand for finding new and efficiency sources for pharmaceutical and functional food products. This research proved that seeds used as traditional remedy had significant antioxidant and antimicrobial activities and they can serve as potential source of bioactive .compounds and are utilized effectively without being wasted.The results will increase application and use-value of the seeds as well as find out a new pharmaceutical material with reasonable and competitive prices for industrial scale production and commercial purposes.

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TÓM TẮT

HOẠT TÍNH KHÁNG OXI HÓA VÀ KHÁNG VI SINH VẬT CỦA CÁC DỊCH CHIẾT TỪ HẠT CÂY CHUỐI HỘT (*MUSA BALBISIANA*)

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Trong bài báo này, một số chiết xuất từ hạt cây chuối hột *Musa balbisiana* được chuẩn bị với một số dung môi như ether dầu hoả, ethyl acetate và butanol. Kết quả cho thấy quá trình chiết có hỗ trợ vi sóng cho hiệu suất cao. Chiết xuất buthanol và ethyl acetate cũng thể hiện hoạt tính kháng oxi hoá tốt (sử dụng phản ứng với gốc tự do DPPH, 1-diphenyl-2-picrylhydrazyl) với giá trị IC_{50} tương ứng là 182,6 và 301,8 µg/mL. Nồng độ ức chế tối thiểu MIC của cao buthanol là 22,5 mg/mL với cả hai vi sinh *E. faecalis* và *S. aureus*. Bên cạnh đó, cao ethyl acetate có giá trị MIC là 7,5 và 3,5 mg/mL cho *E. faecalis* và *S. aureus*. Từ những kết quả của nghiên cứu này, những chiết xuất trên có triển vọng trong những nghiên cứu và ứng dụng trong tương lai.

Từ khóa: hạt cây chuối hột, *Musa balbisiana*, kháng oxy hóa, IC_{50}, kháng khuẩn, MIC.